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UCRL-PROC-202441

Autonomous Pathogen Detection System

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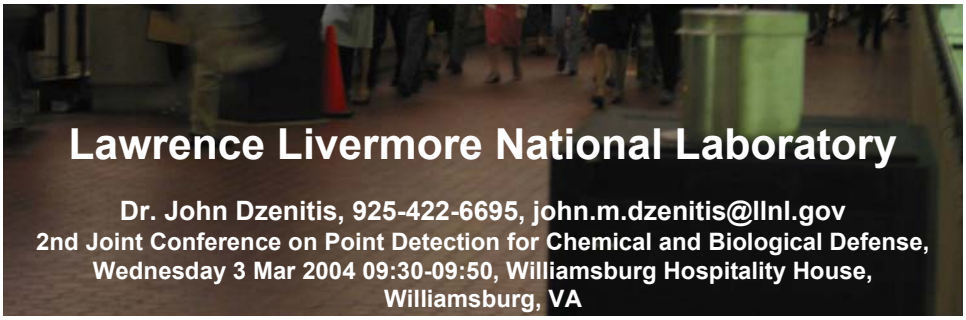
February 20, 2004

2nd Joint Conference on Point Detection for Chemical and Biological Defense
Williamsburg, VA, United States
March 3, 2004 through March 3, 2004



Autonomous Pathogen Detection System

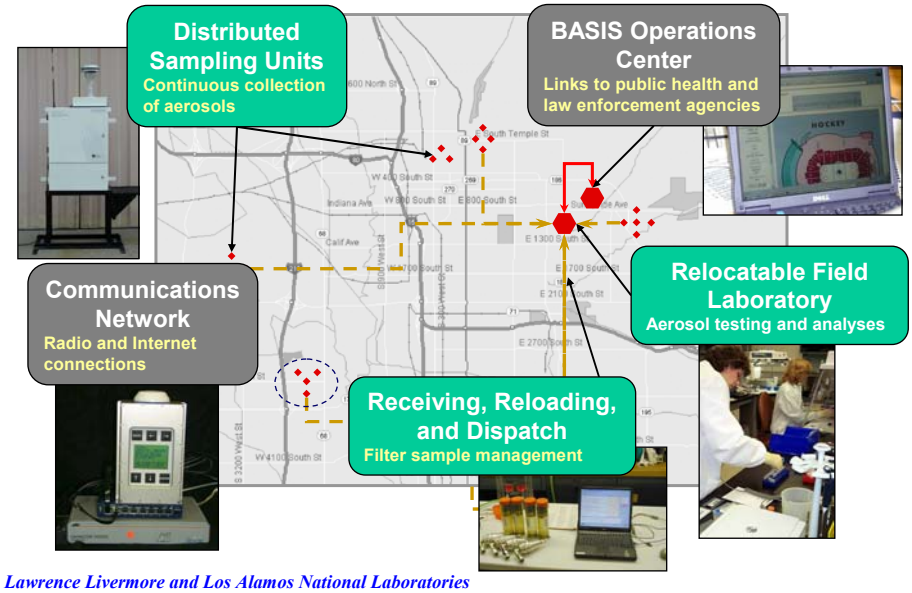
APDS team

- Assays
  - Mary McBride
  - Shanavaz Nasarabadi
  - Sally Smith
  - Venkat Venkateswaran
- Software, control, comm.
  - Bruce Henderer
  - Ujwal Sathyam
  - Dean Hadley
  - Robert Johnson
  - Paul Sargis
- Hardware
  - Ben Hindson
  - Tony Makarewicz
  - Bill Benett
  - Dora Gutierrez
  - Tom Metz
  - George Dougherty
- Project
  - John Dzenitis
  - Bill Colston

This work was performed under the auspices of the U. S. Department of Energy by the University of California, Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48.

Funding from DOE, DHS, and DoD.

BASIS and BioWatch:
Centralized testing of air filters for biological agents



Lawrence Livermore and Los Alamos National Laboratories

Autonomous Pathogen Detection System:
Analysis at collector, networked reporting of results

- Aerosol collection
  - Up to 3,000 Lpm
  - Particle size selection
  - Samples are archived, can be cultured
- Sample preparation
  - Sequential injection analysis platform
  - Flexible and expandable
- Multiplexed detection and identification
  - Bead-based, Luminex™ immunoassay panels
    - bacteria, viruses, protein toxins
    - 11-plex + 4 controls
  - PCR confirmation of DNA sequences
  - Any antibody or sequence can be incorporated
- Data acquisition and control
  - Custom acquisition and analysis software
  - Wireless, Cellular, & Ethernet networking

APDS

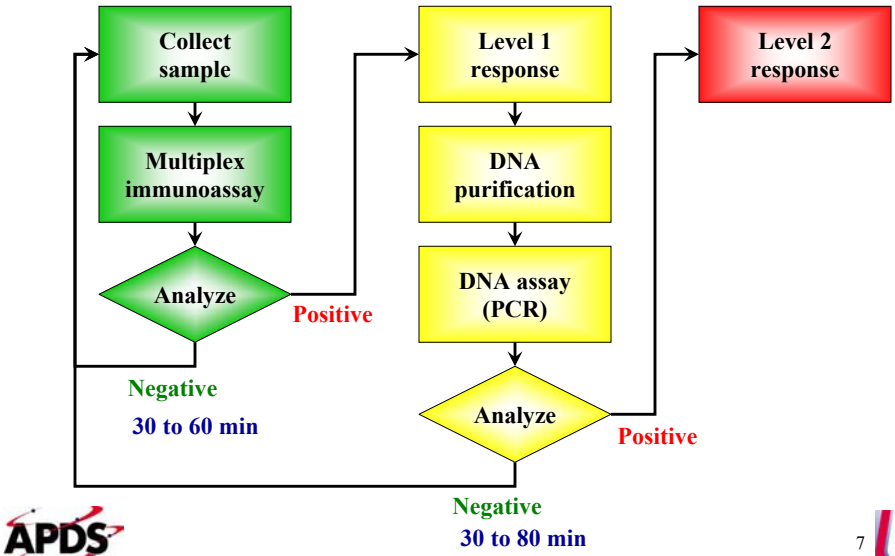
Civilian & base protection differs from the battlefield

- Threat less known
  - Must test for more agents
- Operation is never-ending
  - Operating cost must be lower
- Different impact of alarms
  - Much less tolerance for false positives
- Treating victims *vs.* force protection
  - Some speed can be sacrificed for certainty

The APDS has advantages over the state-of-the-art

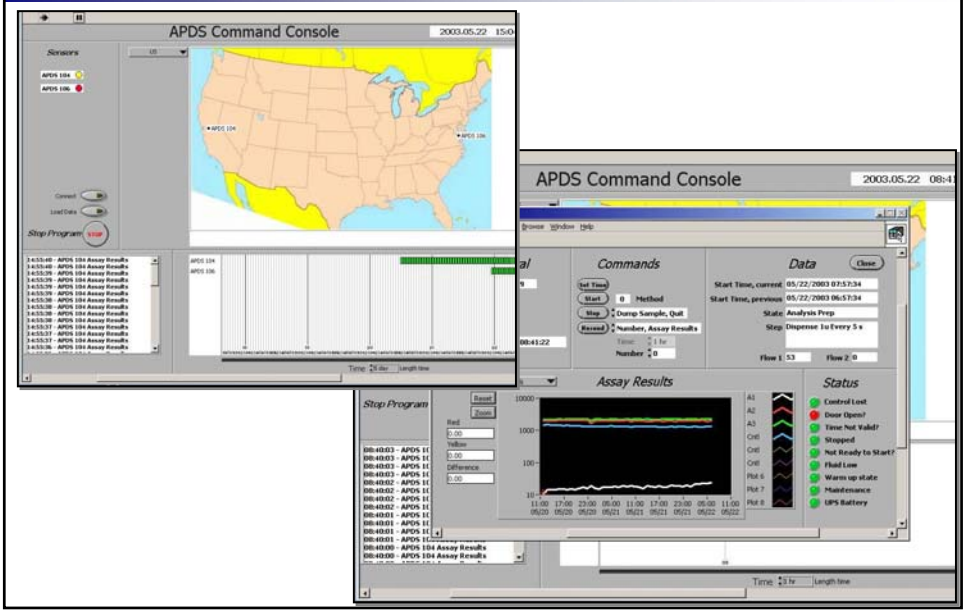
- APDS advantages *vs.* manual filter collection
  - Lower operating cost
  - Shorter time from collection to answer
  - Higher frequency reporting
- APDS advantages *vs.* triggered strip tests
  - Lower operating cost
  - Higher-quality antibody assays
  - PCR assay included
  - Assay upgrade/expansion is simple
- Issues for military applications
  - Ruggedization, equipment cost, speed *vs.* strip test

APDS gives lab-quality answers before a sample could get back to a lab

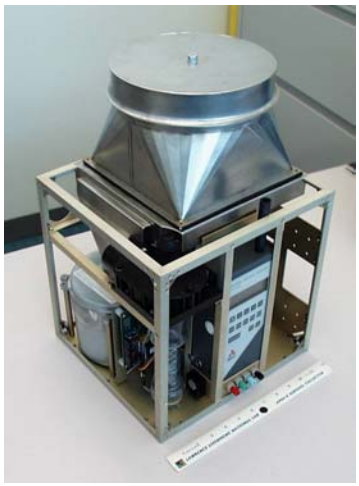


APDS

APDS software and communications allow remote command and control

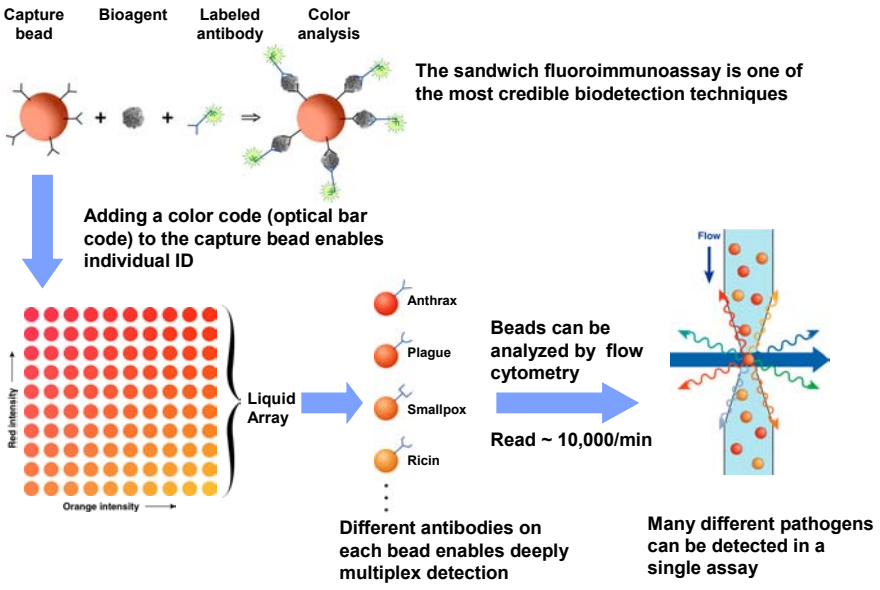


High flow-rate aerosol collection



- 200 – 3,000 Lpm air sample in
- 4 mL liquid sample out
- Multistage
  - Prefractionator cap
  - Virtual impactor
  - Wetted-wall cyclone

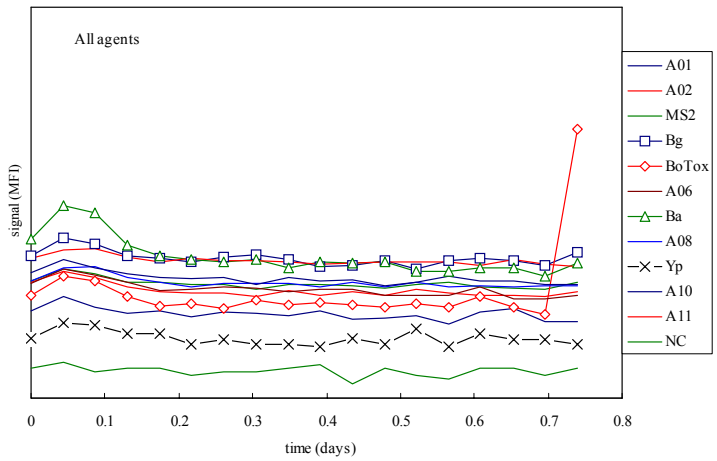
Deeply multiplexed immunoassays with Luminex™



Data-rich signals from flow cytometer are monitored to detect and identify biological agents

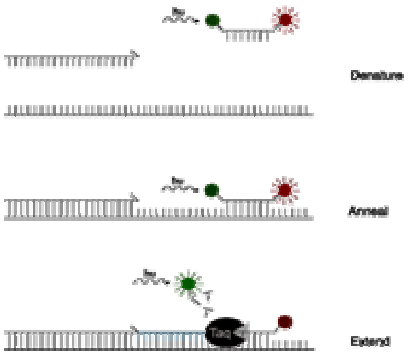
- Fluorescent intensities, numbers of beads, statistics
- Multiplex signals have extra information
- Internal controls are important for confidence
  - Instrument control (detector OK)
  - Fluorescent control (label OK)
  - Antibody control (labeling antibody OK)
  - Negative control (no nonspecific binding)

Example of multiplex immunoassay signals



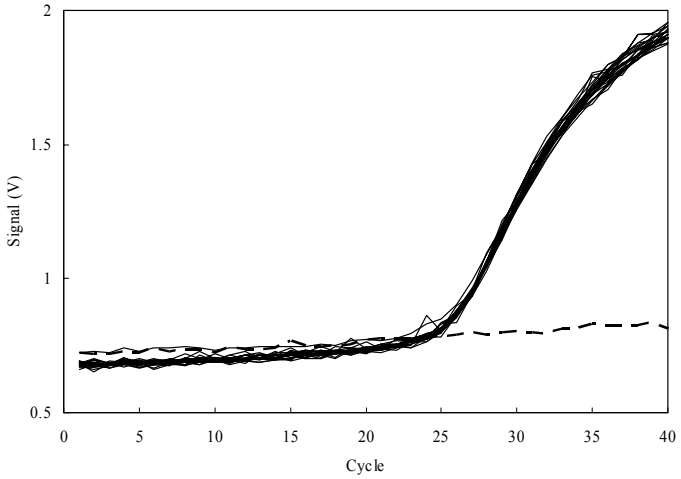
Orthogonal identification using PCR

- Uses DNA instead of protein recognition
  - Looking for different signature, so "orthogonal"
  - Tremendous amplification gives great sensitivity
- TaqMan used for confirmatory PCR

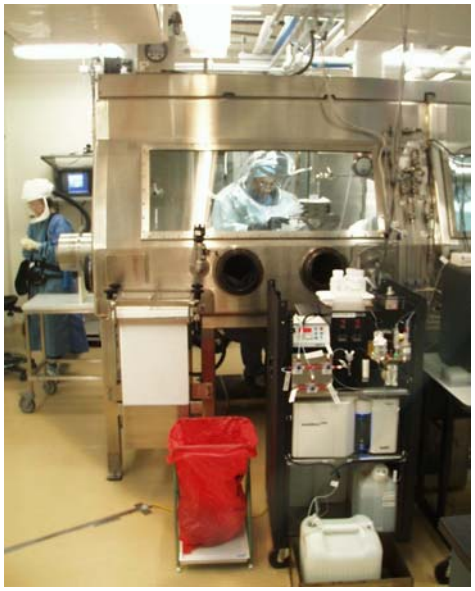


APDS automated PCR is shockingly repeatable

24 consecutive PCR runs & 1 negative control

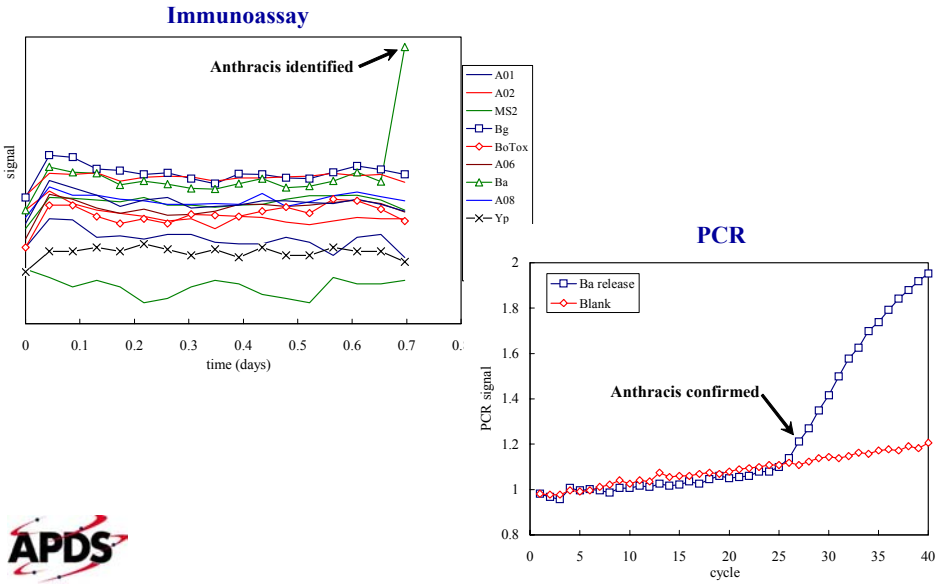


Proven in chamber testing at Dugway Proving Grounds



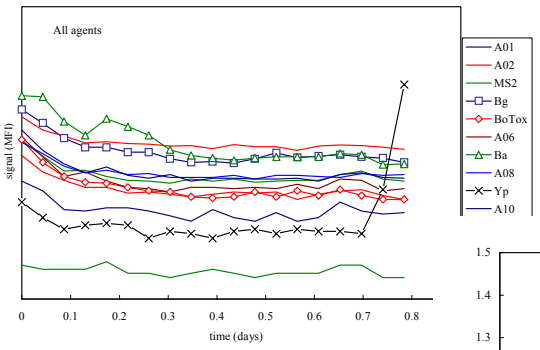
- September 2002
  - Multiplex immunoassay
  - Live-agent releases
    - *B. anthracis* (anthrax)
    - *Y. pestis* (plague)
- September 2003
  - Multiplex immuno. + PCR
  - Killed-agent releases
    - *B. anthracis* (anthrax)
    - *Y. pestis* (plague)
  - Simulant releases
    - Botulinum toxoid, *B. globigii*

Identification and confirmation of a *Ba* release

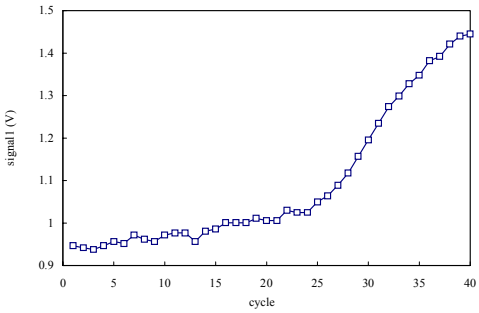


Identification and confirmation of a *Yp* release

Immunoassay

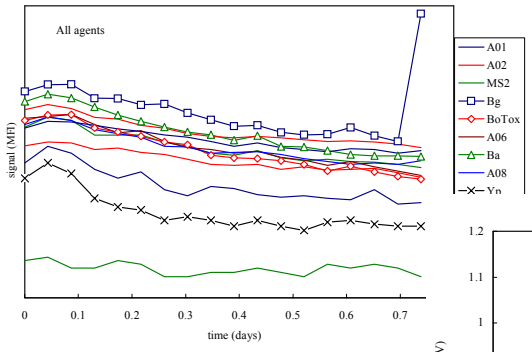


PCR

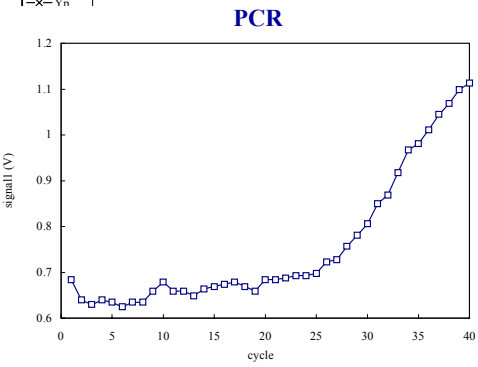


Identification and confirmation of a *Bg* release

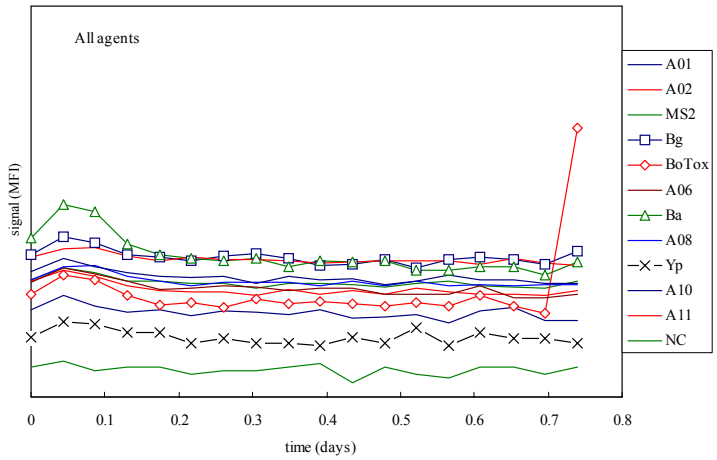
Immunoassay



PCR



Identification of a botulinum toxoid release



APDS

Proven in fully autonomous testing

- Washington DC subway
  - June 2003
  - 1 unit, 7 days
- Albuquerque airport
  - December 2002
  - 2 units, 4 days
- Laboratory runs
  - 24 × 7 for 3 weeks
  - Many shorter runs
- Continuing tests in field

Current work

- Department of Homeland Security
  - Field operation
  - Commercialization
- Department of Defense (Tech. Transition Program)
  - Triggered by early-warning detector (BAWS)